AN OVEVIEW OF BIOEQUIVALENCE

-By
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OUTLINE:

- Definition
- Selection of Reference Product
- Study Designs
- Inclusion and Exclusion Criteria
- Sampling Schedule
- Truncation Procedure
- Fasting and fed studies
- Sample Size Estimation
- Analytes to be measured
- PK parameters to measured
- Bioequivalence Criteria

SPECIAL CONSIDERATIONS

- Light sensitive drugs
- Poor PK fit
- Outlier Testing
- Highly variable Drugs
- Questions.

Definition of Protocol:

A document that describes the objective(s), design, methodology, statistical considerations, and organization of a trial.

QUERY

- In worst case, obtain the following minimum requirements to initiate the protocol.
  - Name of the molecule
  - Type of formulation
  - Regulatory region
  - Literature.

SELECTION OF REFERENCE PRODUCT:

- In general, reference product should always be obtained from the respective country.
- In case of USA submission, obtain Reference Listed Drug (RLD & RS or RS) from Orange Book.
- Always refer final OGD recommendation to have the details of dose to be administered and the study design details.

  NOTE: Always BE studies on the highest strengths are preferred to waive the BE studies on lower strengths .

SELECTION OF REFERENCE PRODUCT:

- **In case of Europe submission, obtain the available highest strength of reference product in the country.**

- **NOTE 1: On safety grounds, lower strengths can also be justified.**
  Sometimes dose may be increased to two times or three times to the actual dose for better quantification of analytes.

- **NOTE 2: Additional BE studies may have to be conducted in consideration to the linearity and applicable regulatory requirements.**

SELECTION OF REFERENCE PRODUCT:

- **In case of Canada, Brazil, Australia, Turkey etc….. Countries submission, obtain the available highest strength of reference product in the respective country.**

- **ROW (rest of the world) market including India, the reference product would be the Innovator’s product.**

NOTE 3: In general, innovator who develops the product will always launch the product globally, therefore the reference product company details will remain same except the country change, due to the change in mfg sites and distribution rights as desired by the Innovator.

STUDY DESIGNS

- Two-way Crossover Design (RT or TR) –The most preferred design.
- Blinded (Preferred for Canada) or unblinded studies are also accepted for BA/BE studies.
- Unblinded –The most preferred one
- Parallel Design - for Long Half-Life drugs.
- Three-way Cross-over design (RT1T2 or T1T2R or T1RT2, R1R2T or R2TR1 or TR1R2)
- Replicate Design (RTRT or TRTR) - The most preferred design for highly variable drugs.
- Partial Replicate Design (or) Reference Scaled Design (TRR or RRT or RTR) - for highly variable drugs.

DRUG SPECIFIC INCLUSION CRITERIA AND EXCLUSION CRITERIA

- **Refer to the product monograph and see the BOX warnings and precautions if any and include all these points as exclusion criteria.**

- **Find out the most prominent adverse events in comparison with that of Placebo and include all these adverse events as exclusion criteria**

- **Find out laboratory abnormalities and include the required specific lab tests to be done at the time of screening or before check-in or at the time of post study laboratory investigations.**

- **Include the routine Inclusion Criteria and Exclusion Criteria in the protocol.**

SAMPLING SCHEDULE:

- Before occurring C_{max} (Absorption phase) at least 3 time points to avoid the first point as $T_{max.}$
- During Distribution Phase (pleatu) at least 3 time points.
- During Elimination Phase at least 4 time points for accurate estimation of Kel.
- At least 10 half-lives washout period to avoid carry over effect.

SAMPLING SCHEDULE:

- In case of MR products- Sampling should be expanded.
    - The purpose is to reduce the frequency of dosing.
    - To obtain maximum therapeutic benefit while maintaining the plasma drug concentration within the therapeutic window over a period of time.

- If the ratio of AUCT/AUC∞ < 80% indicates sampling should be expanded.

- The best possible way of deciding sampling schedule is refer pilot study data. If not available go through the literature. Always look for pictorial presentation of data for better prediction about the product trend.

TRUNCATION:

- Applicable for long half-life drugs (In general >24 hours).
- In general, truncation can be up to 72 hours post dose. May be hours post dose and can be extended to 96, 120 and 144 hours post dose based on the drug specific characteristics.
- Applicable for only conventional dosage forms (IR) but not for MR dosage forms. Why?
- In case of conventional dosage forms 80% of the drug absorption occurs within 72 hours post dose whereas in case of MR products it is not possible to prove due to formulation characteristics as well as due to dose dumping (major constrain).
- Truncation should avoid in case drugs exhibit high variability in distribution and clearance.
- Advantages: Huge savings in terms of Time and Cost.

FAST AND FED STUDIES:

- In general for USA, fast and fed studies are required for conventional and modified oral dosage forms.
- In case of Europe, Canada, Australia and Brazil etc., only Fasting studies are required for conventional oral dosage forms (or as per SmPC posology recommendations) and for modified oral dosage forms fast and fed studies are also required.
- Specifically for Europe and the countries which follow European regulations, multiple dose studies are also required in addition to the fast and fed studies for modified oral dosage forms .
- Question to the regulatory: When single dose studies are more sensitive in detecting formulation differences why multiple dose studies are required (May be informative for drugs exhibiting non-linear or saturation kinetics).

MULTIPLE DOSE STUDIES:

- Obtain the literature after how many days of dosing the steady state is achieved.

- Obtain the literature at what frequency the drug have to be administered to reach the steady state (once daily/twice daily etc.) from the product monograph or SmPC etc.

- Collect minimum three pre-dose (trough concentrations) samples to confirm the steady state achievement.

- Collect the PK samples over steady state dosing interval.

MULTIPLE DOSE STUDIES:

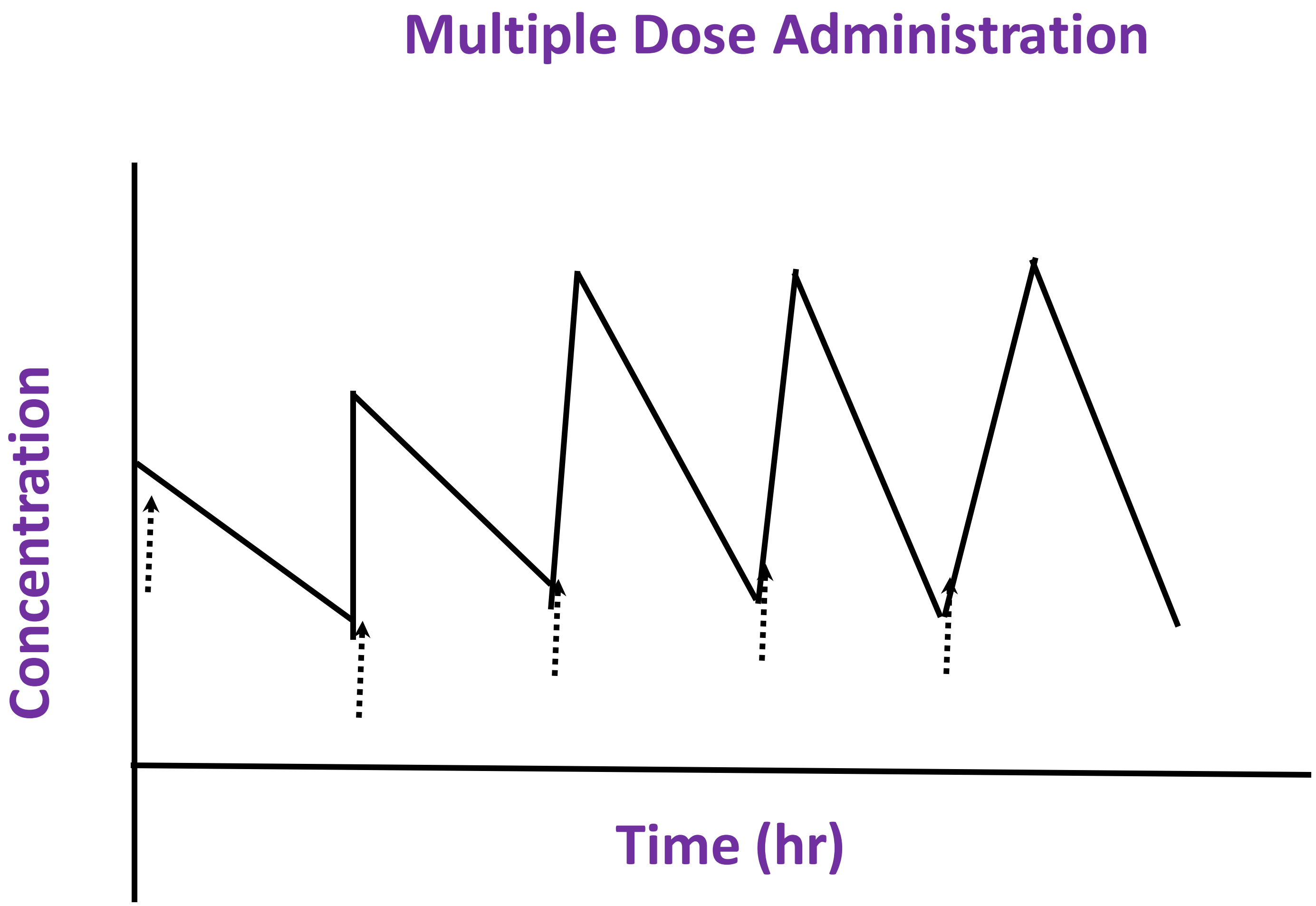
- **Apply linear regression analysis to at least three Predose concentrations to confirm the achievement of steady state.**

- **Check the statistical significance at 5% level of significance. If p-value >0.05, then include those subjects in the final statistical analysis.**

- **If p-value <0.05, then apply the last two days Predose concentrations to obtain the ratio. If the ratio falls within 80.00-125.00%, then include those subjects in the final statistical analysis.**

- **If the ratio falls outside 80.00-125.00%, then exclude those subjects from the final statistical analysis**

Steady state concepts



- Minimum and maximum conc should be within therapeutic window – depends on dose, frequency and $t_{1/2}$
- Depending on dosing frequency and $t_{1/2}$, accumulation occurs
- Degree of accumulation is important for safety assessment purposes

WHEN MULTIPLE DOSE STUDIES REQUIRED:

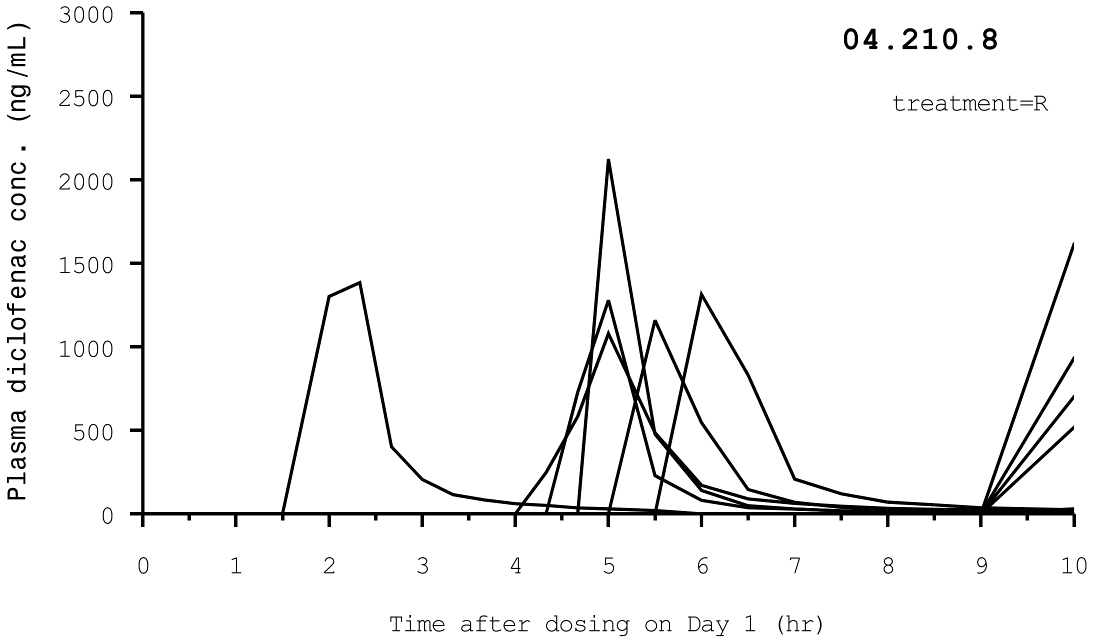
Multiple dose studies in case of…..

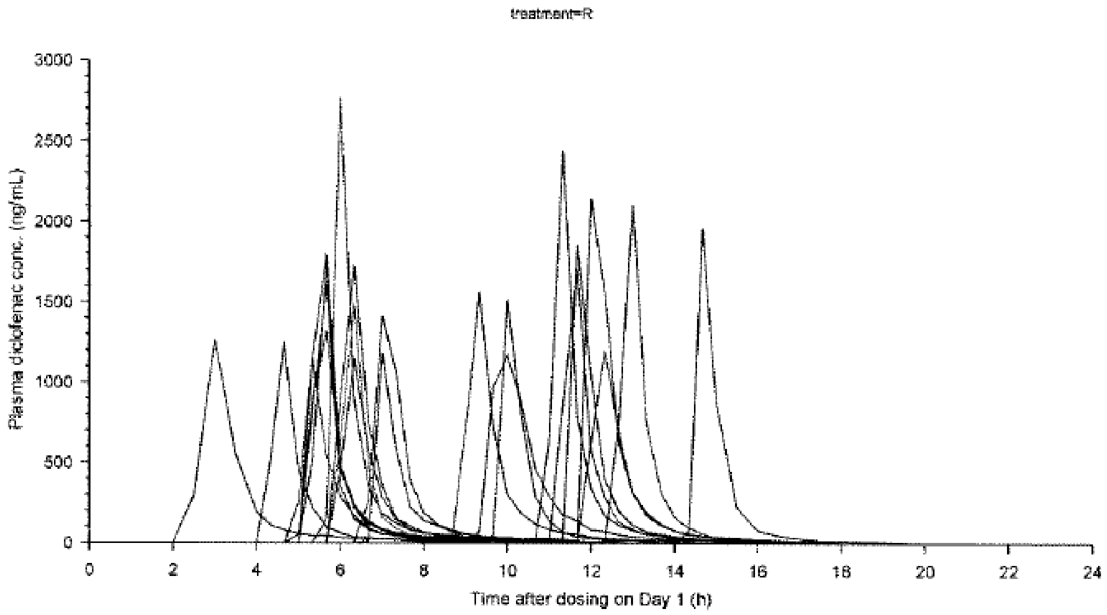
- Drug too potent/toxic for healthy volunteers –patients/ no interruption therapy
- Extended/modified release formulations – accumulation / unexpected behavior
- Non-linear PK at steady state
- Analytical assay sensitivity

FOOD EFFECT:

Food effect due to change in:

- Gastric emptying time
- Acid secretion
- Intestinal motility
- Bile secretion
- Enzyme secretion
- Active absorption process





SAMPLE SIZE ESTIMATION

$ne = [t(\alpha, 2n-2) + t(\beta, 2n-2)]^2 * [CV/(V-\delta)]^2$

where

n- is sample size as per pilot study.

CV- is Coefficient of Variation (intra-subject variability) of that PK parameter which has maximum variability.

V- is Log of Bioequivalence limit (0.8 or 1.25) i.e. 20% difference.

(In general 10% difference is considered. In case of highly variable drugs go for 5% difference of Generic Vs Innovator)

δ- is Absolute value of expected/ observed difference.

α- is level of significance, generally 5%.

β- is probability of Type II error.

Note: The minimum number of subjects that would be acceptable is 12. Care should be taken to avoid any bitter any experience.

Analytes to be measured:

- In general parent should always be quantified and be used for 90% confidence interval approach.
- In case of active metabolites, data should be provided as supportive evidence.
- Follow the regulatory guidance as appropriate.

PK parameters:

Single dose studies : Routine PK parameters of C_{max}, $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, T_{max}, Kel and $T_{1/2}$.

Truncated studies : C_{max}, $AUC_{0\rightarrow 72}$ and T_{max}.

Steady state studies : AUC_t, C_{max}, C_{min}, C_{avg}, T_{max}, swing and %fluctuation

Bioequivalence Criteria

- In general parent should always be used for 90% confidence interval approach.
- In case of active metabolites, data should be provided as supportive evidence.

- Single dose studies : C_{max}, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$.
- Truncated studies : C_{max} and $AUC_{0\rightarrow 72}$.
- Steady state studies : $AUC_{0-\tau}$, $C_{max,}$ and C_{min}
- EU Steady state studies: $AUC_{0-\tau}$, $C_{max,}$ and $C_{\tau ss}$

Bioequivalence Criteria:

USFDA:

- **90% CI should be within 80.00-125.00 % for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ irrespective of the type of formulation.**
- **NTI: 90% CI should be within 80.00-125.00 % for C_{max}, AUC_{0-t} & $AUC_{0-\infty}$, 95% upper confidence bound <0.0000 for C_{max}, AUC_{0-t} & $AUC_{0-\infty}$ and $S_{WR} \leq 2.5*S_{WT}$.**

EUROPE:

- **IR formulations: 90% CI should be within 80.00-125.00 % for C_{max} and AUC_{0-t}.**

- **MR formulations: 90% CI should be within 80.00-125.00 % for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.**

- **NTI: 90% CI should be within 90.00-111.00 % for AUC and on case to case basis 90% CI will be tightened for C_{max}.**

Bioequivalence Criteria:

BRAZIL ANVISA:

- **90% CI should be within 80-125 % for C_{max} and AUC_{0-t} irrespective of the type of formulation.**

CANADA:

- **90% CI should be within 80.0-125.0% for AUC_{0-t} and ratio should be within 80.0-125.0% for C_{max} irrespective of the type of formulation.**
- **NTI: 90% CI should be within 90.0-112.0% for AUC_{0-t} and for C_{max} 90% CI should be within 80.0-125.0% for $C_{max.}$**

JAPAN:

- **90% CI should be within 80-125 % for C_{max} and AUC_{0-t} irrespective of the type of formulation.**

RETENTION SAMPLES:

S. No	Country	Retention Quantity	Retention Period
1.	USA	Five times all of the release tests. An upper limit of 300 units is sufficient	5 years from the date the bioavailability or bioequivalence study was completed.
2.	WHO	Sufficient quantity as per national regulations	Equal to the shelf-life of the products where applicable, plus one year.
3.	EUROPE	At least, two occasions, of the full analytical controls on the batch in accordance with the IMP dossier submitted for authorisation to conduct the clinical trial.	Reference and retention samples of investigational medicinal product, including blinded product should be kept for at least two years after completion or formal discontinuation of the last clinical trial in which the batch was used, whichever period is the longer.

RETENTION SAMPLES:

S. No	Country	Retention Quantity	Retention Period
4.	BRAZIL	The number of units of the test and the reference drug products that are to be retained enough to repeat the study.	These samples must be stored in proper conditions to preserve the original characteristics of the products up to their expiry date
5.	South Africa	A sufficient number of retention samples of both test and reference products used in the bioequivalence study,, in order to allow **re-testing** if required by the MCC. **Hence twice quantity for re-analysis is sufficient**	should be kept for one year in excess of the accepted shelf-life, or two years after completion of the trial or until approval, whichever is longer.
6.	China	The testing unit shall keep samples of the test and reference preparations according to relevant requirements	The test drug should be kept in the sample for 2 years after the drug is approved for marketing

RETENTION SAMPLES:

S. No	Country	Retention Quantity	Retention Period
6.	Canada	Specific quantity is not mentioned, however records respecting the shipment, receipt, disposition, return and destruction of the Drug should maintain accurately. **Note: It is appropriate to keep the retention quantity to repeat the study (if query raises), since Health Canada does not audit the facilities**.	25 years
7.	India	Each reserve sample should consist of a quantity sufficient to carry out twice all the in-vitro and in-vivo test required during BA/BE study.	Three years after conduct of the study or one year after the expiry of the expiry of the drug, whichever is earlier.

Requirements for intravenous liposomal products:

Comparative investigation of pharmacokinetics (including tissue distribution), toxicology and pharmacodynamics.

Non-Clinical Studies

Non-clinical pharmacodynamic studies

- Interaction between liposomes and target cells or other cells.
- Demonstration of the similarity in pharmacodynamic response using appropriate in-vivo models and at various dose levels chosen taking into account the sensitivity of the model.

Non-clinical pharmacokinetic studies

- Appropriate species and models to investigate the in-vivo release of the drug from liposomes.

Requirements for intravenous liposomal products:

- **Dose to be investigated**
  - Single and multiple dose studies at different dose levels may be needed.
  - Doses should be chosen based on blood levels observed at therapeutic doses in man.
- **Analytes to be measured**
  - both the unencapsulated drug and the encapsulated drug should be investigated if feasible.

- ***Toxicological studies***

In general, toxicity studies may not be needed**. Based on** nature of any toxicity produced by the product, appropriate organ function tests may be required to support equivalence in the context of known target organ toxicity.

-

Requirements for intravenous liposomal products:

Clinical Studies

- **Dose to be investigated**:
  - Highest strength if linearity is demonstrated.
  - linearity with encapsulated, unencapsulated, as well as total drug substance through literature support.
  - non-linearity, demonstration of bioequivalence at the highest and lowest doses.

Design considerations

- Single dose in healthy subjects,
- Single dose study in patients, if not feasible in healthy population.
- Multiple dose studies in patients, if single dose study not feasible in patients.

Requirements for intravenous liposomal products:

Analytes to be measured:

- Total, encapsulated and unencapsulated drug substance
- Quantification of at least one metabolite regardless of its pharmacological activity.

Pharmacokinetic parameters to be measured and reported:

C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, K_{el}, V_d, Cl

Acceptance criteria:

Similarity should be demonstrated for the total, encapsulated and unencapsulated drug. Generally, the 90% confidence intervals C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ ratios should be within 80 - 125%.

SPECIAL CONSIDERATIONS:

- **Light sensitive drugs:** All clinical and analytical procedures should be carried out under yellow monochromatic light.
- **Endogenous substances:** Baseline corrections should be applied to represent the actual drug concentrations.

  PK parameters:

  C_{max}, $AUC_{0\rightarrow t}$ and T_{max} will be reported for baseline uncorrected data. C_{max}, $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \infty}$, T_{max}, Kel and $T_{1/2}$ will be reported for baseline corrected data.
- Due to physiological fluctuations of endogenous levels of Endogenous substances, the $AUC_{0-\infty}$ might not be properly characterized in some subjects which is only due to improper estimation of Kel.

SPECIAL CONSIDERATIONS:

- Poor pharmacokinetic fit: $R^2 < 0.8$. How? (Not defined in any regulatory guidance)

  Obtain slope from Log conc vs time curve.

  K_{el}= -2.303 × Slope. Slope generally obtained from Log linear phase of the elimination. If best fit line did not observed, leads to $R^2 < 0.8$ nothing but poor PK fit.

- Alternative to poor PK fit, $AUC_{0\text{-}t} / AUC_{0\text{-}\infty}$ >80%.

- When Kel is not available, the inter related parameters of $AUC_{0\text{-}\infty}$ ($AUC_{0\text{-}t} + C_t/K_{el}$) and $T_{1/2}$ (0.693/Kel) can't be calculated.

SPECIAL CONSIDERATIONS:

Outliers: The reasons for outliers is…

- Variable nature of the molecules in ADME.
- Protocol violation with regards to the study procedures.
- Formulation characteristics (often with MR products due to dose dumping)
- Poor metabolizers Vs Extensive metabolizers .
- Significant Food effect (often come across with Delayed Release formulations)
- In general, removing outliers from the data analysis is not accepted.
- In case outliers found in the data, results with including and excluding outliers should be presented.

SPECIAL CONSIDERATIONS:

Handling of Outliers:

- **In case USA submission, re-dosing study should be conducted (20% of subjects from the original study or a minimum of 3 to 5 control subjects) to prove the scientific validity and to increase the acceptance by the regulatory agencies.**
- **Re-dosing study acceptance criteria.: Mean± 3SD criteria is the most preferred one as it covers 99.7% of the data.**
- **This acceptance criteria should be generated from the original study for ln-transformed data by excluding the outlier subject.**

SPECIAL CONSIDERATIONS:

Handling of Outliers:

- Removing outlier subject's data is not accepted for Brazil – ANVISA submission.
- 5% of outliers can be removed from the data in case of TPD-Health Canada submission provided the outlier is identified for both C_{max} and AUC.
- Removing outlier subject's data for TEST product is not accepted for UK MHRA, TGA – Australia submission. However it is accepted for REFERENCE product where AUC is less than 5% of reference medicinal product geometric mean AUC (which should be calculated without inclusion of data from the outlying subject).

Special Considerations:

Highly variable drugs: Drugs exhibit ISCV >30% within reference product.

- **Impact on sample size: Due to the high variability more subjects are needed to reach the bioequivalence (BE) limits of 80 -125% in case of HVDs.**
- **e.g. T/R ratio ± 5%, power 80%:**

  CV = 20 % n=19 *, CV = 40 % n=66 * , * according to Diletti, 1991.
- **To avoid the difficulties in the volunteer recruitment, the preferred designs are full replicate or partial replicate designs to produce more data using less number of subjects for better conclusion.**
- **Global variance: The combined variability of both Test and Innovator.**

Special Considerations:

Highly variable drugs:

- **Switchability: Determines variability when a patient switched from Generic to Innovator or vice versa.**
- **More suitable designs to find outliers.**
- **Increase the acceptability of the studies by the regulatory agencies.**
- **% HVDs generally have a wide therapeutic window as they are safe and effective despite the high variability.**
- **Conclusion: As HVDs have a wide therapeutic window and the number of required subjects should be feasible, a widening of acceptance limits based on reference variability was implemented by authorities (reference-based scaling approach).**

Special Considerations:

Highly variable drugs: First criteria:

In case of $(\mu_T - \mu_R)^2 - \theta^* S^2_{WR} \leq 0.0000$, alternatively

$$\frac{(\mu_T - \mu_R)^2}{\sigma^2_{WR}} \leq \theta$$

Where $\theta = (\log(1.25)/\sigma W_0)^2$ and $\sigma W_0 = 0.25$.

S_{WR} (σ^2 WR) : Within-subject standard deviation (SD) of the reference product

μ_T : Geometric mean of test product

μ_R : Geometric mean of reference product.

Second criteria: Ln-transformed point estimates should be within 80.00-125.00%.

Reference-scaled BE approach: Comparison of US and EU

	US	EU
basic approach	scaled average BE (SABE)	average BE with expanding limits (ABEL)
method applicable if	Intra-CV > 30 % = $S_{WR} > 0.294$	Intra-CV > 30 % = $S_{WR} > 0.294$
within-subject variability (S_W)	based on reference only	based on reference only
Applicable for	AUC and Cmax	only Cmax
Differences: regulatory constant (k) regulatory limit ($б_O$)	$k = 0.893$ $б_O = 0.25$	$k = 0.76$ $б_O = 0.294$
Formula	$\left(\bar{Y}_T - \bar{Y}_R\right)^2 - \theta s_{WR}^2$	$[U, L] = \exp\,[\pm k \cdot s_{WR}]$
Acceptance criteria		
T/R ratio of geomean should be within	80.00 - 125.00 %	80.00 – 125.00 %
BE assessment based on	95% upper confidence bound ≤0	90 % confidence limits within the given range
no further widening of limits if intra-CV ≥ 50%	n.a.	yes maximum 69.84 – 143.19 %
pre-specification in protocol	✓	✓
clinical justification of wider acceptance limits	n.a.	✓
high CV not caused by outliers	n.a.	✓

SPECIAL CONSIDERATIONS:

Add-on study studies:

- Accepted for Canada and Europe but not in USA.

- As a result of random variation or a larger than expected relative difference, there is no guarantee that the sample size as calculated will pass the standards. If the study is run with the appropriate size and the standards are not met, the sponsor may add more subjects (a minimum of 12). The same protocol should be used (i.e., same formulations, same lots, same blood sampling times, a minimum number of 12 subjects, etc).

- The level of confidence should be adjusted using the Bonferroni procedure. The t-value should be that for p=.025 instead of 0.05.

SPECIAL CONSIDERATIONS:

Sequential designs

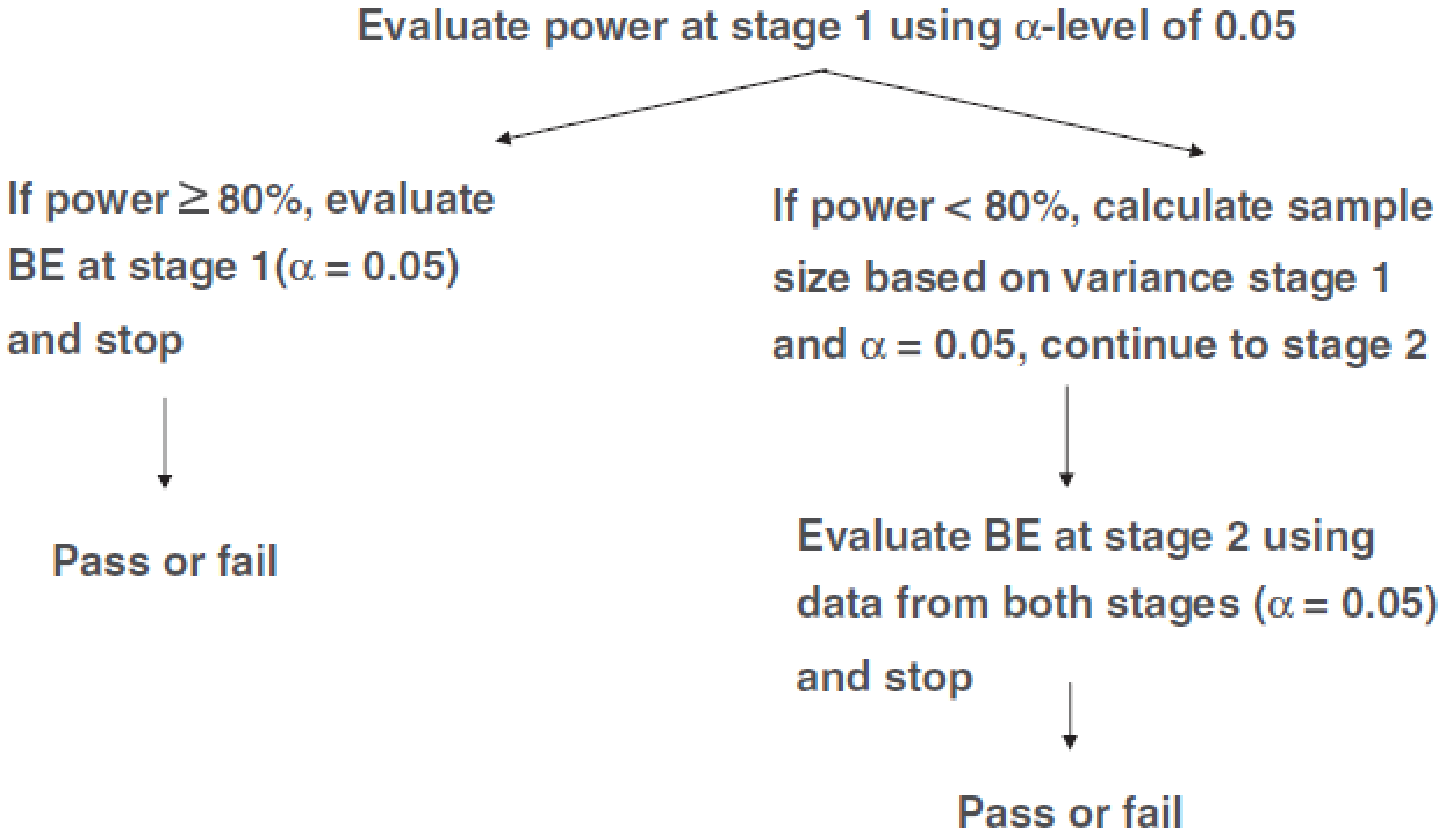
- In general study will start with 24 subjects, if the and the study will be conducted. study did not meet the BE criteria with adequate power (at least 80%), the sample size will be estimated based obtained ISCV and with the required power.
- Data Analysis will be done while adjusting the α (level of significance.)
- Initial α (level of significance) is 0.05. while combining the two studies the overall α 0.025.

Studies with groups or cohorts:

- In general, studies requires huge sample size will be conducted in equal groups or cohorts due to the difficulty in recruitment process and lack of bed capacity and other infrastructure as appropriate.
- ANOVA will be presented while adding the group effect (Group by treatment interaction will be assessed).

SPECIAL CONSIDERATIONS:

Adaptive sequential designs: D.Potvin et al, Method A



Evaluate power at stage 1 using α-level of 0.05

If power ≥ 80%, evaluate BE at stage 1($\alpha = 0.05$) and stop

Pass or fail

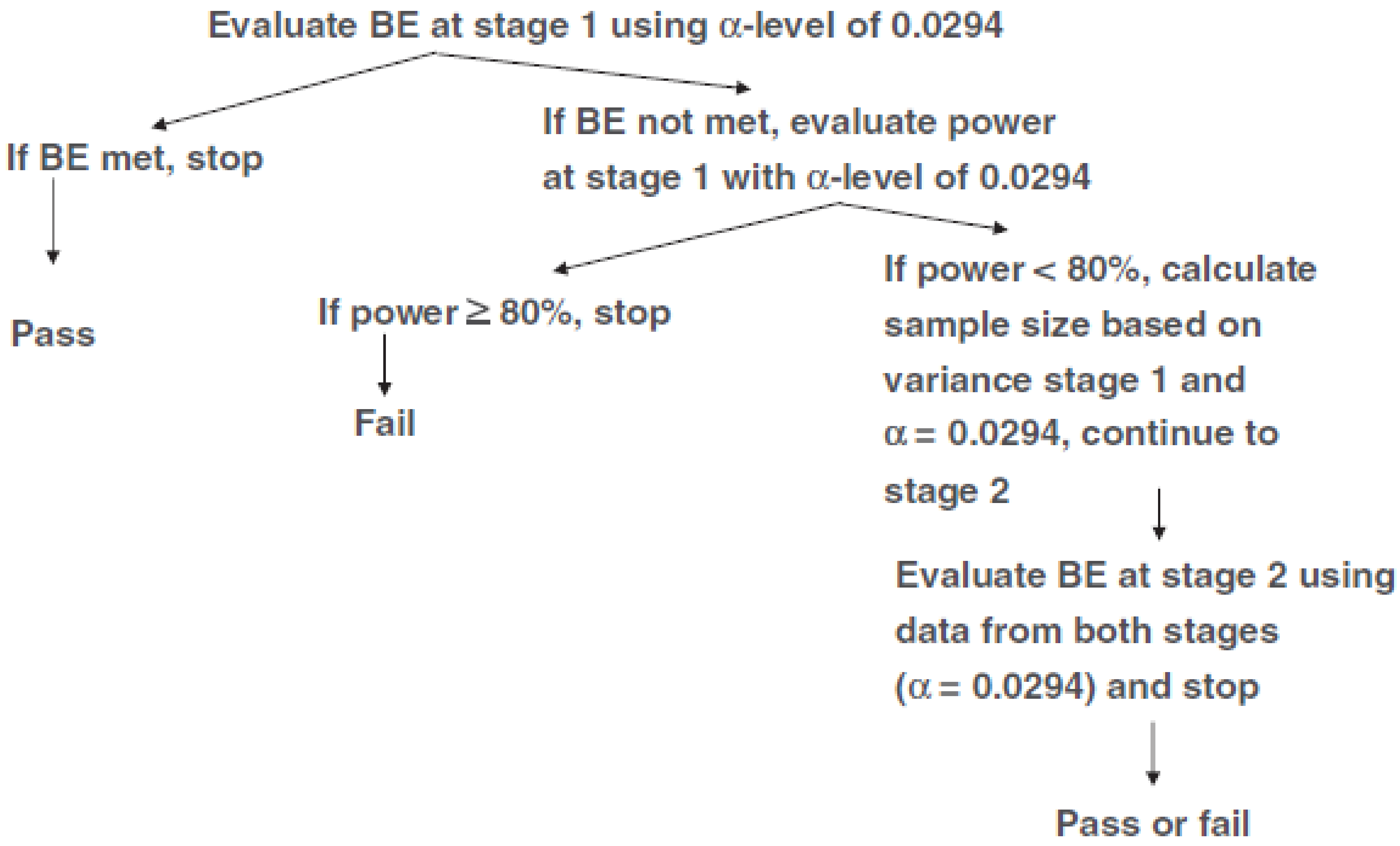
If power < 80%, calculate sample size based on variance stage 1 and $\alpha = 0.05$, continue to stage 2

Evaluate BE at stage 2 using data from both stages ($\alpha = 0.05$) and stop

Pass or fail

SPECIAL CONSIDERATIONS:

Adaptive sequential designs: D.Potvin et al, Method B



Evaluate BE at stage 1 using α-level of 0.0294

If BE met, stop

Pass

If BE not met, evaluate power at stage 1 with α-level of 0.0294

If power $\geq$ 80%, stop

Fail

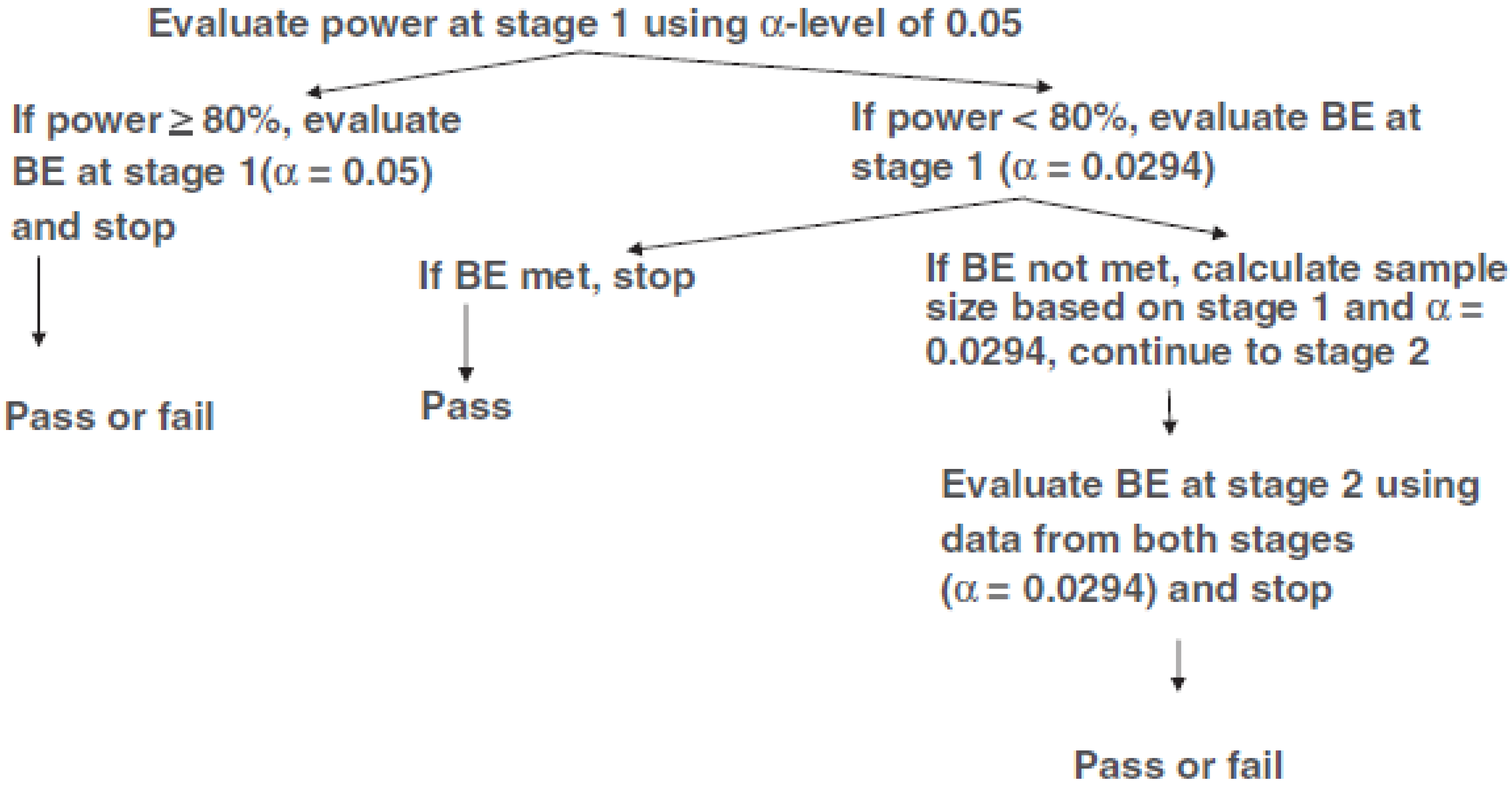
If power < 80%, calculate sample size based on variance stage 1 and $\alpha = 0.0294$, continue to stage 2

Evaluate BE at stage 2 using data from both stages ($\alpha = 0.0294$) and stop

Pass or fail

SPECIAL CONSIDERATIONS:

Adaptive sequential designs: D.Potvin et al, Method C.



Evaluate power at stage 1 using α-level of 0.05

If power ≥ 80%, evaluate BE at stage 1(α = 0.05) and stop

Pass or fail

If power < 80%, evaluate BE at stage 1 (α = 0.0294)

If BE met, stop

Pass

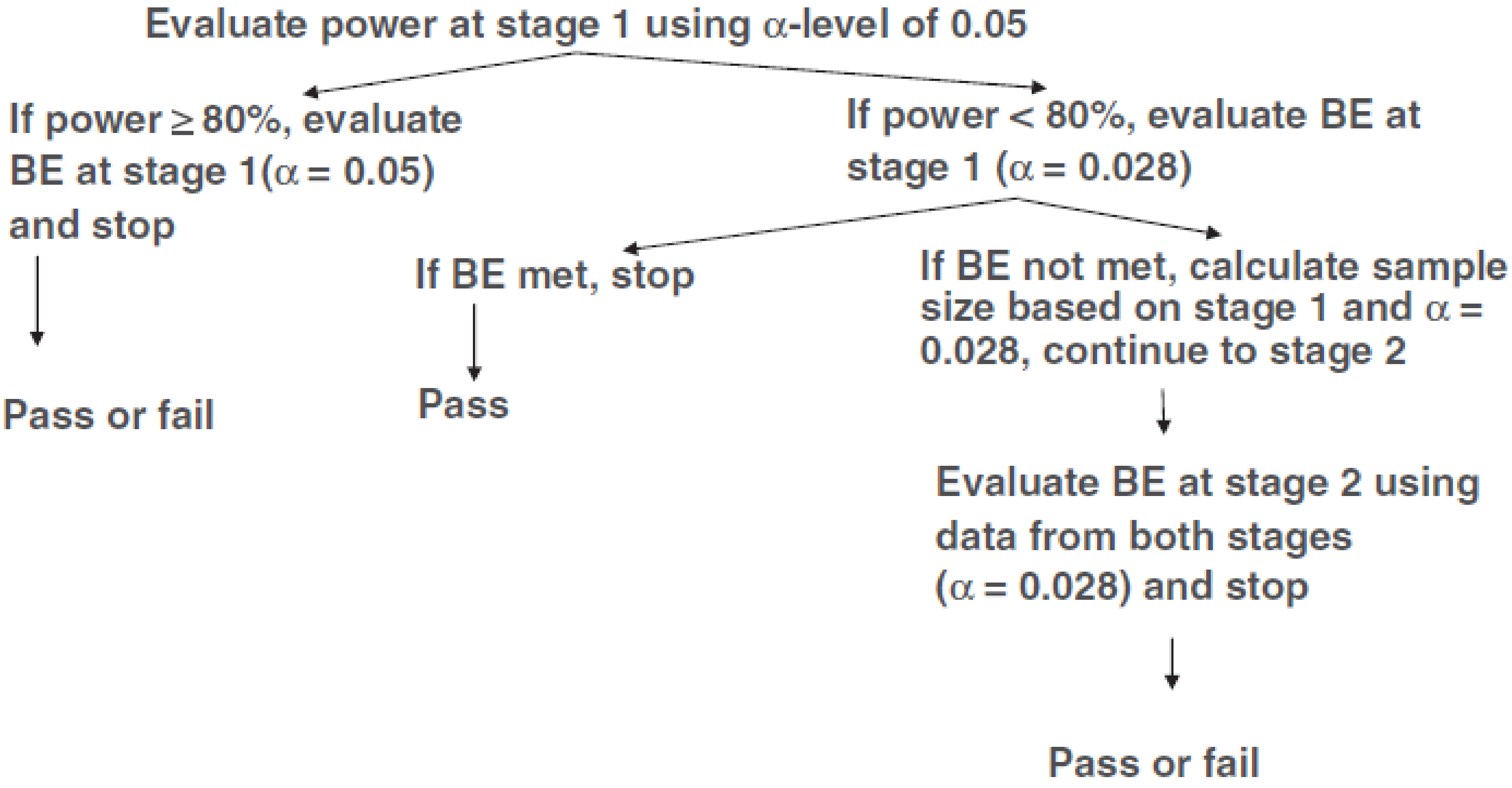
If BE not met, calculate sample size based on stage 1 and α = 0.0294, continue to stage 2

Evaluate BE at stage 2 using data from both stages (α = 0.0294) and stop

Pass or fail

SPECIAL CONSIDERATIONS:

Adaptive sequential designs: D.Potvin et al, Method D.



Evaluate power at stage 1 using α-level of 0.05

If power $\geq$ 80%, evaluate BE at stage 1($\alpha = 0.05$) and stop

Pass or fail

If power < 80%, evaluate BE at stage 1 ($\alpha = 0.028$)

If BE met, stop

Pass

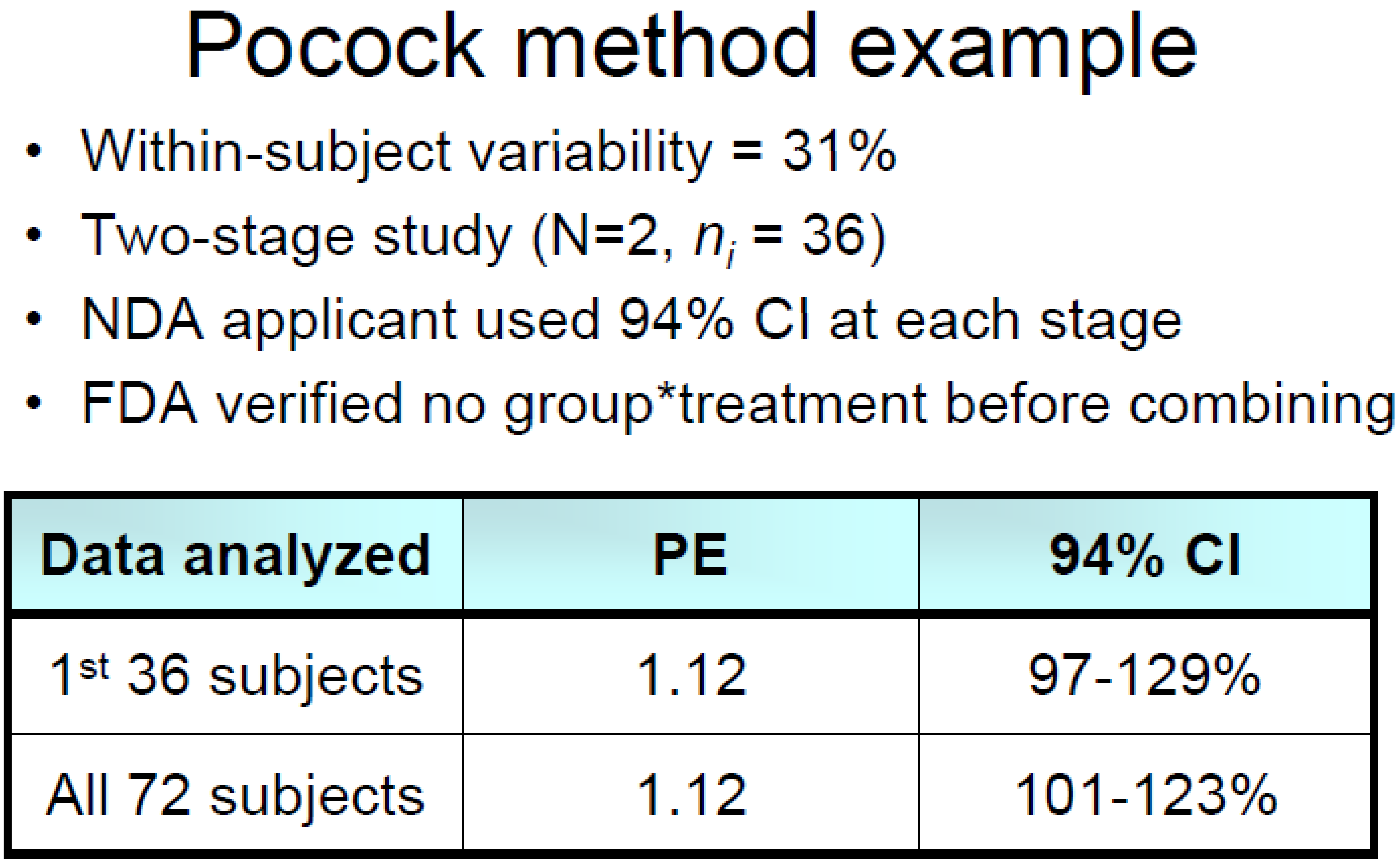
If BE not met, calculate sample size based on stage 1 and $\alpha = 0.028$, continue to stage 2

Evaluate BE at stage 2 using data from both stages ($\alpha = 0.028$) and stop

Pass or fail

SPECIAL CONSIDERATIONS:

Adaptive sequential designs: Pocock. et al



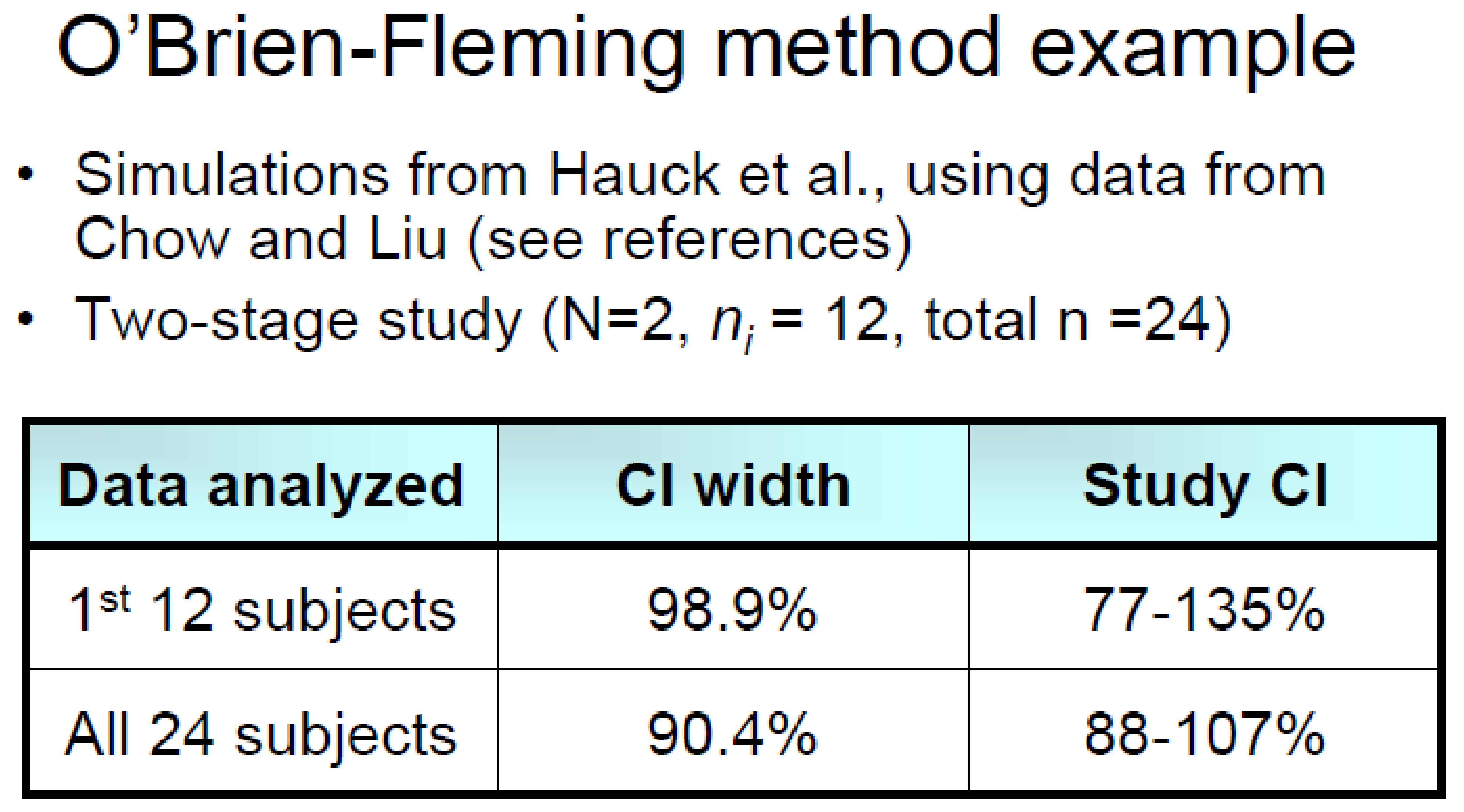
Pocock method example

- Within-subject variability = 31%
- Two-stage study (N=2, n_i = 36)
- NDA applicant used 94% CI at each stage
- FDA verified no group*treatment before combining

Data analyzed	PE	94% CI
1st 36 subjects	1.12	97-129%
All 72 subjects	1.12	101-123%

SPECIAL CONSIDERATIONS:

Adaptive sequential designs: O’Brien-Fleming. et al



O’Brien-Fleming method example

- Simulations from Hauck et al., using data from Chow and Liu (see references)
- Two-stage study (N=2, n_i = 12, total n =24)

Data analyzed	CI width	Study CI
1st 12 subjects	98.9%	77-135%
All 24 subjects	90.4%	88-107%

Handling statistical analysis for studies conducted in two groups

— If a large number of subjects are to be recruited, the study site may not be large enough to accommodate the subjects.

— In these situations, the study subjects may be divided into two cohorts.

— Each cohort is used to assess the comparative products individually, as might be done in two separate studies.

— Typically, the **two cohorts** are of approximately equal size.

— The final assessment is based on a combination of both groups.

Handling statistical analysis for studies conducted in two groups

— The totality of data is analyzed with a new term in the ANOVA, a Treatment-by-Group interaction term.

— If interaction is large **(p <0.1)**, as tested in the ANOVA, then the groups statistically should not be combined.

— However, if at least one of the groups individually passes the confidence interval criteria, then the test product might be acceptable.

— If interaction is **not statistically significant (p > 0.10)**, then the confidence interval based on the pooled analysis, after dropping the interaction term, will determine acceptability.

— It is an advantage to pool the data, as the larger number of subjects increases power and there is a greater probability of passing the BE confidence interval, if the products are truly bioequivalent.

Handling statistical analysis for studies conducted > two groups

- If there is no interaction**(p >0.1),**, the data should be pooled.
- If interaction is evident **(p <0.1),** it is implied that at least one group is different from the others.
- The remaining groups may then be tested for interaction. If there is no interaction, the data should be pooled.
- If there is interaction, the aberrant group may be omitted, and the remaining groups tested, and so on.
- In rare cases, it may not be obvious which group or groups are responsible for the interaction. In that case, more statistical treatment may be necessary, and a statistician should be consulted.

Handling statistical analysis for studies conducted > two groups

- In any event, if any single group or pooled groups (with no interaction) passes the BE criteria, the test should pass.
- If a pooled study passes in the presence of interaction, but no single study passes, one may still argue that the product should pass, if there is no apparent reason for the interaction.
- For example, if the groups are studied at the same location under the identical protocol, and there is overlap in time among the treatments given to the different groups, as occurs often, there may be no obvious reason for a significant interaction.
- Perhaps, the result was merely due to chance. One may then present an argument for accepting the pooled results.

Handling statistical analysis for studies conducted > two groups

- In the presence of interaction, verify demographics similarity; background variables like concomitant medication usage, disease history, and/or comorbid conditions; or baseline disease severity etc.
- Perform an ad-hoc analysis for outliers in the groups.
- Ignore the group-by-treatment in the statistical model, if the clinical study takes place at one site, all study subjects have been recruited from the same enrolment pool, if the subjects have similar demographics, and all enrolled subjects are randomly assigned to treatment groups.
- **Model 1: GRP, SEQ, GRP∗SEQ ,SUBJ(GRP∗SEQ), PER(GRP), TRT, GRP∗TRT.**
- **If the GRP∗TRT term is not significant (P > 0.10), then reanalyze the data using Model 2.**
- **Model 2: GRP, SEQ, GRP∗SEQ, SUBJ(GRP∗SEQ), PER(GRP), TRT.**

QUESTIONS:

- **Why 240 mL of water in particular?**
- Sufficient volume is required to swallow tested dosage form. It can 150 mL (Canada and Japan), 200 mL (Brazil) and varies country to country. Volume of water does not play any scientific role.

- **What is 90% Confidence Interval?**
- The limits within which the parameter estimation is called confidence interval. 90% CI means, if you repeat an experiment, it reproduces the same results 90 times and 10 times is may differ. It is obtained from TOST procedure where $\alpha=0.05$.

- **About range 80 – 125 % for BE Criteria.**
- It is obtained based on the ±20 rule and Ln-transformation of data to ensure the normality i.e. to observe the symmetry.

QUESTIONS:

- **Why only Cmax & AUC considered for BE and not Tmax?**

- **Cmax & AUC are directly represents the rate and extent of drug absorption respectively, which are very important PK parameters, directly correlates the safety and efficacy, whereas Tmax is only part of rate of drug absorption.**

- **Tmax is considered for some regulatory agencies (Brazil) and is considered when there is particular importance of a therapeutic benefit. Moreover it is a discrete variable and hence non-parametric 90% CI calculated and reported.**

QUESTIONS:

- Why some studies are conducted under fasting conditions only, and not performed fed conditions?
- It is based on the regulatory requirements and drug specific characteristics and sometimes for safety reasons.

- How to select the RLD for Europe, Canada and Australia?
- RLD from should be obtained from the respective county.

- How to select the dose strength?
- Based on the linearity and RLD status & RS or RS.

QUESTIONS:

- **Is it the BMI criteria and age limits are based on study to be conducted or based on the regulatory requirement?**
- **It is based on the regulatory requirement. In general 18-30kg/m^2 is accepted for all countries.**
- **Why PROC MIXED is used for Canada instead of PROC GLM in two-way design?**
- **PROC MIXED procedure can handle the data accurately in all conditions like presence of missing sample data and imbalanced data.**
- **Is there is any case of Parallel design study concluded with re-dosing study?**
- **Handle such cases by keeping the criteria of range i.e. min and max values.**

QUESTIONS:

- **Is there any variation between “comparative oral bioavailability study” and “oral bioequivalence study”?**
- **Yes, bioavailability (Cmax and AUC) can be compared e.g. IV Vs oral, IM vs oral, oral Tab Vs oral Cap., etc without any criteria, but for BE, 90% CI should be within 80-125%.**

- **Is it “180-day exclusivity” applicable for Indian regulatory too, besides the USA?**
- **180-day exclusivity is applicable for only USA but not applicable in India and is as per the country specific law.**

QUESTIONS:

- Why apple sauce sprinkle studies are conducted?
- Apple sauce sprinkle studies are conducted when the label mentioned that drug is to be administered while sprinkling on apple sauce.

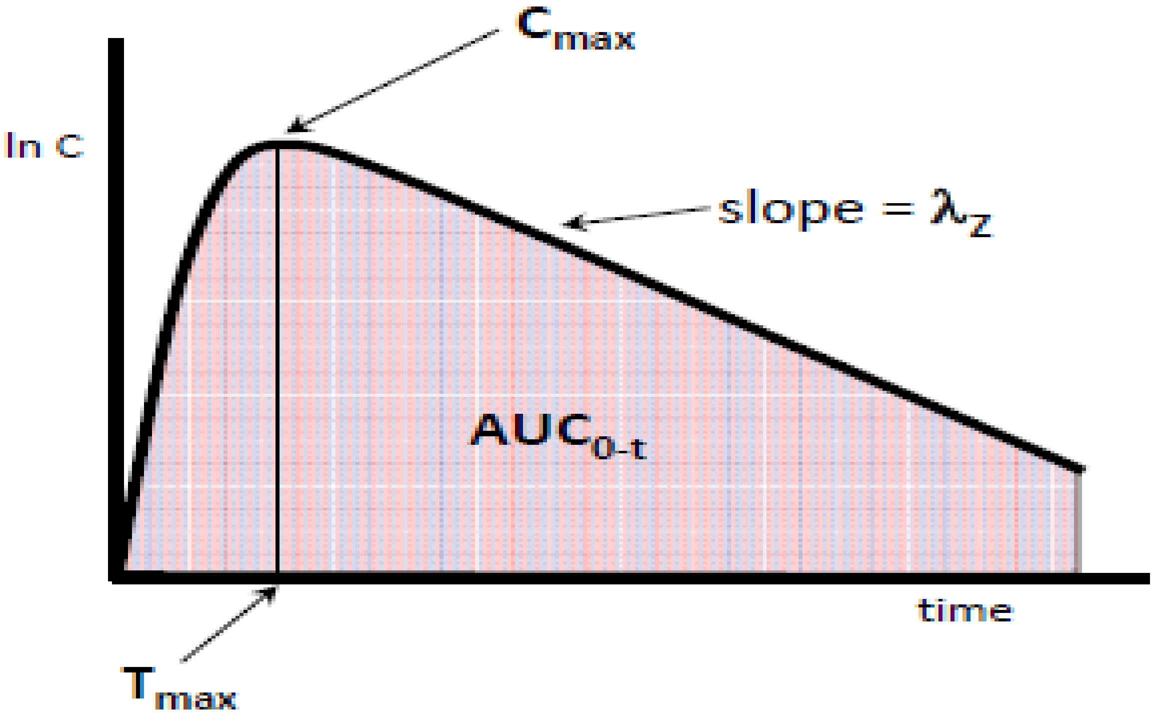
- Apple sauce sprinkle studies are conducted to prove that there is no drug interaction with apple sauce.

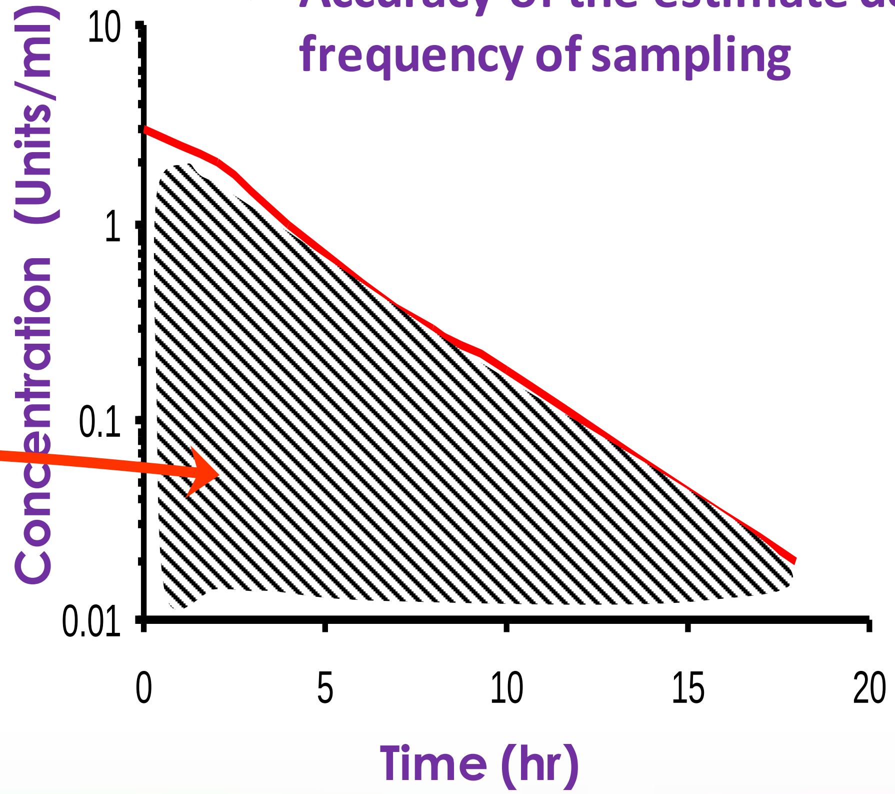
- The drug can be sprinkled on any other soft food provided label should allow the same.

QUESTIONS:

Calculation of PK parameters.

- ✓ A quantitative measure for exposure from dosing time to time ‘t’
- ✓ An important parameter in PK
- ✓ $AUC_{(t)}$ and $AUC_{(inf)}$
- ✓ Determined by trapezoidal method
- ✓ $AUC_{(inf)} = AUC_{(t)} + C_t/k$
  Units: Conc*t (mg/L * h)
- ✓ Accuracy of the estimate depends on frequency of sampling

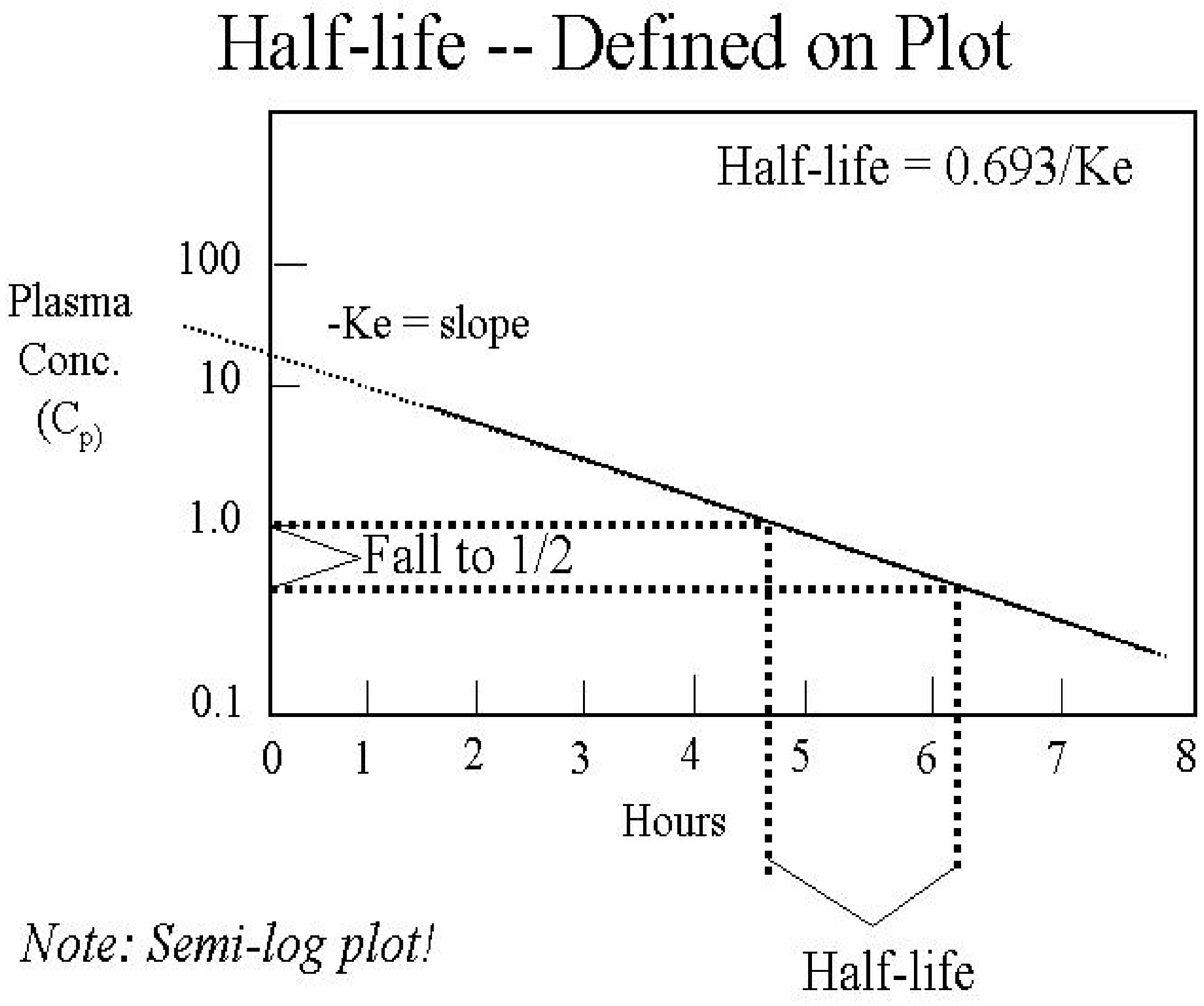




Area Under the Curve (AUC)

QUESTIONS:

Calculation of PK parameters.



$$k_e = \frac{\left(\ln y - \ln \frac{y}{2}\right)}{T_{1/2}}$$

$$k_e = \frac{\ln y - \ln y + \ln 2}{T_{1/2}}$$

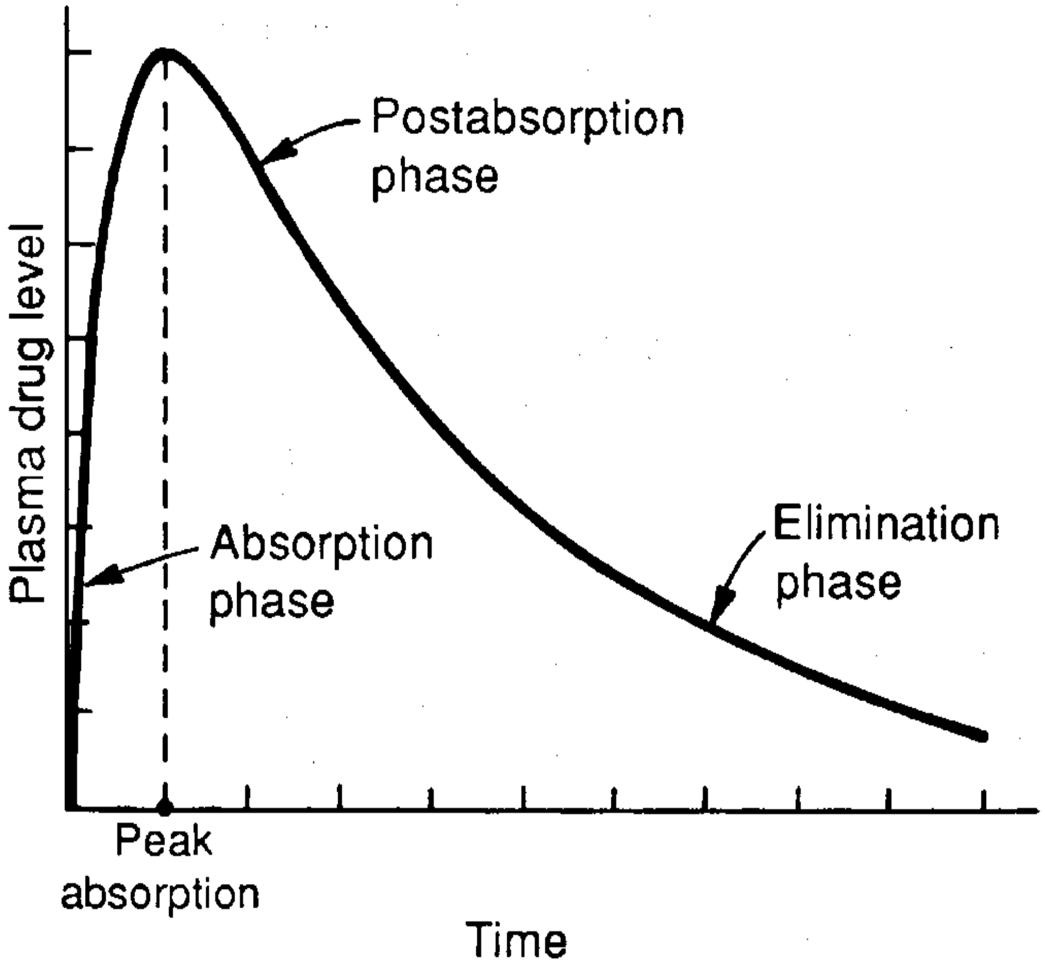
$$k_e = \frac{\ln 2}{T_{1/2}}$$

$$k_e = \frac{0.693}{T_{1/2}}$$

QUESTIONS:

5x the elimination ½ life = time at which the drug is "completely" (97%) eliminated from the body

- 1x ½ life - 50% of the original drug removed
- 2x ½ life - 75%
- 3x ½ life - 87.5%
- 4x ½ life - 93.75%
- 5x ½ life - 96.875%



QUESTIONS:

Graphs linear & semi-log.

Linear and semi-log plots (pictorial representation) are presented is just to understand product in better way.

Untransformed and lntransformed data.

Ln-transformation of data is required to ensure the normal distribution of data i.e. to observe the symmetry.

Most pharmacokinetic parameters have a skewed distribution (log transformation normalizes the distribution).

No need to represent untransformed and is presented just for information.

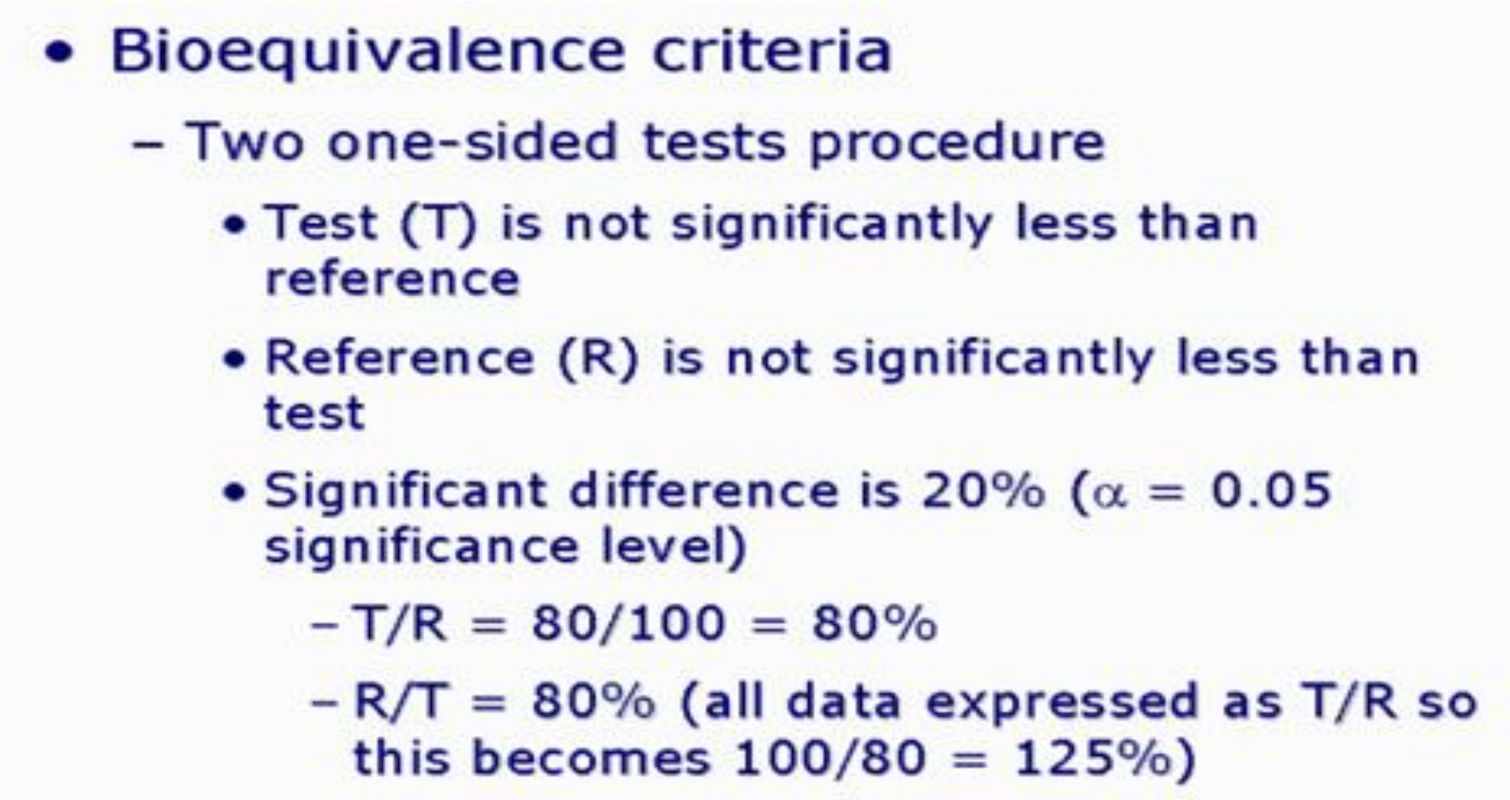
10% level of significance and 5% level of significance.

5% level of significance is considered as a consumer risk which evaluates potential confounding effect of factors including Period, Subject, Treatment (formulation) whereas the Sequence effect evaluated at 10% level of significance.

QUESTIONS:

Generation of bioequivalence range and power calculation.

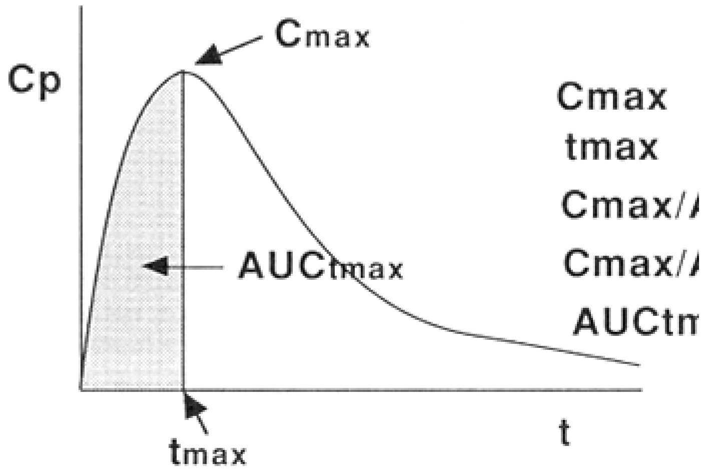
Scaled average approach. – **It is obtained based on the ±20 rule. In general 80% power is considered for BE studies. However , as long as the 90% CI within 80-125%, the study can be submitted irrespective of the power obtained.**



- Bioequivalence criteria
  - Two one-sided tests procedure
    - Test (T) is not significantly less than reference
    - Reference (R) is not significantly less than test
    - Significant difference is 20% ($\alpha = 0.05$ significance level)
      - T/R = 80/100 = 80%
      - R/T = 80% (all data expressed as T/R so this becomes 100/80 = 125%)

QUESTIONS:

Partial areas (Bi-layer formulations or Molecule specific)



Partial areas is considered when there is particular importance of a therapeutic benefit.

Urine data concepts.

Urine collection studies are performed when the given drug is not sensitive enough to detect in the plasma/serum, moreover the drug excreted through urine.

QUESTIONS:

Randomization.

Randomization is done in general to avoid the bias.

When do we calculate V_d and F.

V_d and F can be calculated based on the regulatory requirement like China and Korea, but these are secondary parameters and will not play any role in the evaluation of BE.

First point C_{max} concepts and factors affecting First point C_{max}.

First point C_{max} raises questions of bias in the estimation of C_{max} because of insufficient early sampling times.

Collection of blood samples at an early time point, between 5 and 15 minutes after dosing, followed by additional sample collections (e.g., two to five) in the first hour after dosing is usually sufficient to assess peak drug concentrations.

QUESTIONS:

Replicate designs advantages and disadvantages.

Scientific advantages:

- Comparison within-subject variances T and R
- Indicate whether T exhibits lower or higher within-subject variability
- More information (performance/Subject *Formulation interaction)
- Reduce number of subjects

QUESTIONS:

Replicate designs advantages and disadvantages.

Disadvantages:

Bigger commitment volunteers

More administrations per subject

More expensive

QUESTIONS:

For Modified Release drug formulations (PK & stats considerations for different regulatory regions).

An extended release drug product is a dosage form that allows a reduction in dosing frequency and reduces fluctuations in plasma concentrations when compared to an immediate release dosage form. Extended release products can be formulated as capsules, tablets, granules, pellets, or suspensions.

In general for modified release products, the following studies are required for any given regulatory agency: (1) a single-dose, fasting and fed studies comparing the highest strength of the test with the Reference drug.

Routine PK and statistical analysis is required. Sometimes partial areas need to be calculated based on the recommendation from the regulatory agency.

QUESTIONS:

Linear and Non-linear pharmacokinetics concepts regarding AUC.

For drugs which exhibit linear kinetics, a single-dose fasting and fed studies comparing the highest strength is sufficient to waive the BE studies with lower strengths based in-vitro data.

For drugs which exhibit non-linear kinetics, for each and every strength required BE study (Amoxi-Clav).

Housing time (Checkout period).

In general housing up to 24 hours is sufficient.
If the sampling frequency is high then 48 hours is suggested.
Housing can be increased as long as possible based on the study design (Potassium) and safety (Aripiprazole) aspects.

QUESTIONS:

Sample analysis form (Withdrawn dropouts & absent for the periods).

In general samples from subjects who complete all the study periods will have to be analyzed.

Samples from withdrawn/dropout subjects due to AEs will have to be analyzed to establish relation between the drug exposure and the observed Adverse Event.

Samples from withdrawn/dropout subjects due personal reasons need not to be analyzed.

Vital signs.

In general vital signs should be measured at before dosing, frequently at the maximum drug exposure (around the Tmax of a drug) and at least once daily in the elimination.

Any additional vital signs or lab tests and the frequency of measuring vital signs may differed in accords to the drug nature considering the safety.

QUESTIONS:

Outlier Testing & Topics: Studentized Residuals Test Procedure.

Studentized residuals are defined for each observation, i = 1, 2, 3, - - - n as an ordinary residual divided by an estimate of its standard deviation:

$$r_i = \frac{e_i}{s(e_i)} = \frac{e_i}{\sqrt{MSE\,(1-h_{i,i})}}$$

Where, e_i is the difference between ordinary residual. i.e., $e_i = y_i - \hat{y}_i$, i=1, 2, - - - - n

where $\hat{y}_i$ is regression equation (y = a+bx)

s is standard deviation

$h_i = X (X^T X)^{-1} X^T$ (the quantity of leverage or design),

where X is design matrix $X = \begin{bmatrix} 1 & x_1 \\ \vdots & \vdots \\ 1 & x_n \end{bmatrix}$

QUESTIONS:

Outlier Testing & Topics: Studentized Residuals Test Procedure.

Formula for Lund’s method:

$$
\begin{aligned}
R_{0} &= t_{(n-p-1)}(1-\alpha/n) \\
&\times [(n-p)/(n-p-1+t^{2}_{(n-p-1)}(1-\alpha/\mathrm{n}))]^{1/2}
\end{aligned}
$$

where n is number of observations

p is model degrees of freedom

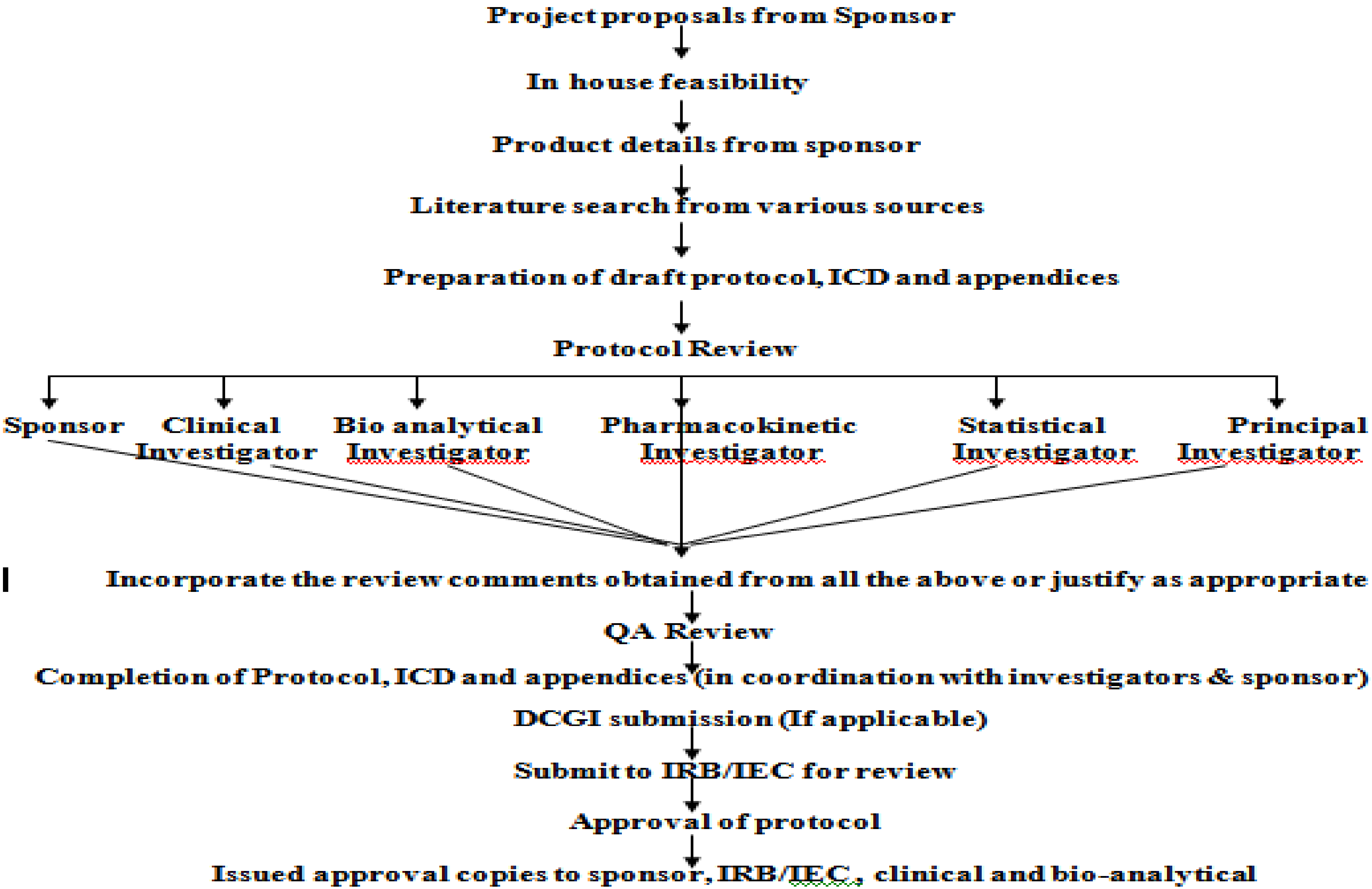
α is level of significance

t is t-distribution

Studentized Residuals Test Procedure.

- Studentized Residuals Test Procedure and Lund’s outlier methods are used combinedly used to declare statistical outliers.
- The critical values (table value) are being calculated using Lund’s formula.
- The calculated values obtained from Studentized Residuals Test (generally calculated for absolute values, eg: Cmax values for test and reference).
- If calculated value (obtained from Studentized Residuals Test)**>** critical value (obtained from Lund’s formula), outlier will be declared.
- If calculated value (obtained Studentized Residuals Test)**<**critical value (obtained from Lund’s formula) no outlier will be declared.

QUESTIONS: Flow chart for protocol initiation to EC approval.



Project proposals from Sponsor

In house feasibility

Product details from sponsor

Literature search from various sources

Preparation of draft protocol, ICD and appendices

Protocol Review

Sponsor

Clinical Investigator

Bio analytical Investigator

Pharmacokinetic Investigator

Statistical Investigator

Principal Investigator

Incorporate the review comments obtained from all the above or justify as appropriate

QA Review

Completion of Protocol, ICD and appendices (in coordination with investigators & sponsor)

DCGI submission (If applicable)

Submit to IRB/IEC for review

Approval of protocol

Issued approval copies to sponsor, IRB/IEC, clinical and bio-analytical

QUESTIONS:

How to select design partial replicate and full replicate.

Full replicate (TRTR | RTRT),
Partial replicate (TRR | RTR | RRT)

Partial replicate: + within subjects (reference)

Full replicate: + within subjects (reference, test) and the global within-subject variability may be estimated.

Comparison of within-subject variability obtained per treatment in case of NTIs is possible with full replicate design.

THANK YOU